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BY

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(Contribution from Wisconsin Agricultural Experiment Station)

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. XXXII, No. 8 : : : : Washington, D. C., April 15, 1926

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1926

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Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

Subscription price: Domestic, $4.00 a year (two volumes)
Single numbers, 20 cents
Foreign, $5.25 a year (two volumes)
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RELATION OF GROWTH IN THE POTATO TUBER TO THE POTATO-SCAB DISEASE[1]

By HURLEY FELLOWS[2]

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INTRODUCTION

That the common potato scab is a parasitic disease was proved by Thaxter (*17*)[3] in 1891, who called the causal organism *Oospora scabies;* but Güssow (*6*), in 1914, transferred the organism to the genüs Actinomyces. Previous to the time of Thaxter's discovery, growers had held several theories as to the cause of potato scab, and had sought in vain for satisfactory methods of control. Since the demonstration of the parasitic nature of the scab, investigations concerning it have dealt chiefly with control methods, with the effect of environmental factors on the production of the disease, and with the cause of resistance. Other investigations have been made of the physiology and taxonomy of the organism.

The experiments reported in this paper were begun as a result of observations made by the writer at the Wisconsin Agricultural Experiment Station while working on the relation of soil temperature to the amount of scab produced on tubers (*9*). It became evident that there was an intimate relation between the rate of growth of the potato tuber and the amount of scab which developed. The tubers which grew most vigorously and became the largest were most severely affected, whereas the small tubers showed only a slight infection. It was also noted that the original seed piece never developed scab lesions, even when planted in soil which was heavily inoculated with the scab parasite. Further observations of several hundred tubers infected with scab revealed the fact that the disease was more severe at the basal end of the tuber than in the apical region. At first thought this might seem to contradict the previous contention that scab development is dependent on growth, unless the tuber, unlike other stems, increases in size more rapidly at the basal end than in the apical region. There was also the possibility that the basal end might be more susceptible to scab than the apical region, or that the tuber might be more susceptible when small than when larger. Further consideration indicated that the tendency for scab localization might be explained on the basis of a time factor, since all observations showed that the base of the tuber possessed the oldest surface tissue. It was thus possible that the disease had been present on the basal end for a longer time and as a result had spread over a larger area.

[1] Received for publication May 26, 1925; issued March, 1926.

[2] The writer makes grateful acknowledgment to L. R. Jones and H. H. McKinney for suggestions in the course of the investigation herein reported. This work was done during the years 1922 and 1923 in partial fulfillment of the requirement for the degree of doctor of philosphy at the University of Wisconsin.

[3] Reference is made by number (italic) to Literature cited, p. 781.

These considerations suggested that the development of scab might be dependent to a large extent on the growth of the host in somewhat the same manner as that suggested by Keitt (*11*) in connection with the increased susceptibility of the young growing leaves and fruit of the apple to *Venturia inaequalis*. Another case of similar nature has been reported by L. K. Jones (*7*) in connection with the immunity of the mature tissue of the raspberry canes to anthracnose. These observations, together with many other known instances of a similar nature, would seem to justify an attempt to determine whether the same is true in the case of potato scab.

A search through the literature showed that little work has been done on the exact manner in which the potato tuber increases in size. Little has been shown, also, regarding the mode of infection by *Actinomyces scabies* and the relative susceptibility of the different parts of the tuber or of the tuber as a whole during different stages of its development. Accordingly, experiments were undertaken to determine the mode of tuber development with especial regard to the method of enlargement, the formation of stomata, lenticels, epidermis, and cork, and the time of tuber setting. The study of stomata, lenticels, epidermis, and cork were made on various portions of single tubers of various sizes. The mode of infection by the organism was given attention. The histology of the lesions was studied in order to determine whether the organism or its products come into direct contact with the more actively dividing cells of the external parts of the tuber and if so, the effect of such contact on these developing tissues.

SOME PHASES OF TUBER GROWTH

METHOD OF STUDY

The studies made of growth processes in the potato tuber were conducted in the field at Madison, Wis., during the summers of 1921 and 1922. These experiments were not designed with a view to finding out all the phases of tuber development from the beginning until growth entirely ceased, but were intended primarily to demonstrate the method of enlargement of tubers from about the time they were one-tenth mature until growth was over. Cytological studies were restricted to those portions of the tuber in which scab might be produced—that is, to the epidermal and corky coverings and the cells immediately beneath. Since tubers vary in shape according to variety, two types were selected which represented fair extremes of length, namely, the Irish Cobbler, which is round, and the Early Ohio, which is elongated (fig. 1). When the tubers were 1 or 2 inches in diameter, the soil was carefully removed from about them and each was marked with India-ink lines. These lines or bands were about 5 millimeters in width and were drawn from the base to the apical end of each tuber. A short line of equal width was made at the growing point in such a way as to intersect the long line at right angles, thus making a cross at the apical end, as shown in Figure 1, C and D. The soil was then replaced, and after varying periods, extending from four to seven days, the soil was again removed and observations made, the spread of the ink band being an index to the amount of growth (pl. 1). Direct measurements were also made in many cases.

In addition to the above-mentioned procedure, a microscopic examination of the marked and unmarked tubers was made to note the location of stomata and lenticels and to observe the method of formation of the latter. Further observations for the same purpose were made on tubers grown in the greenhouse. A microscopic examination was also made of the protective corky and epidermal layers to determine if any difference existed in these layers in different portions of the tuber.

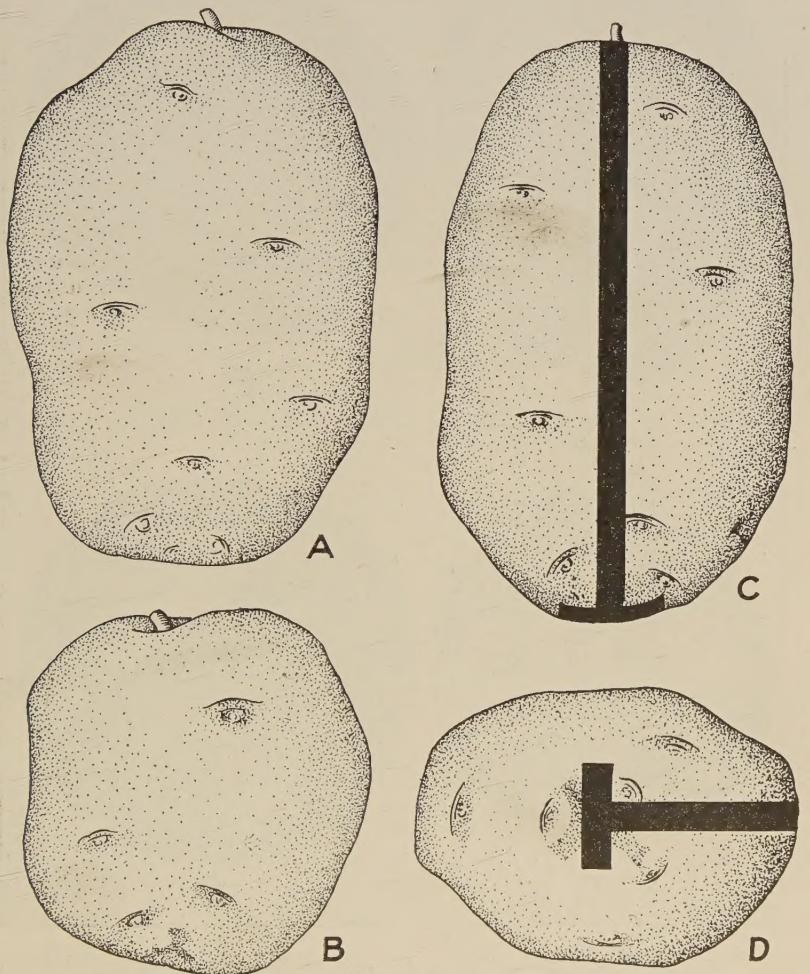
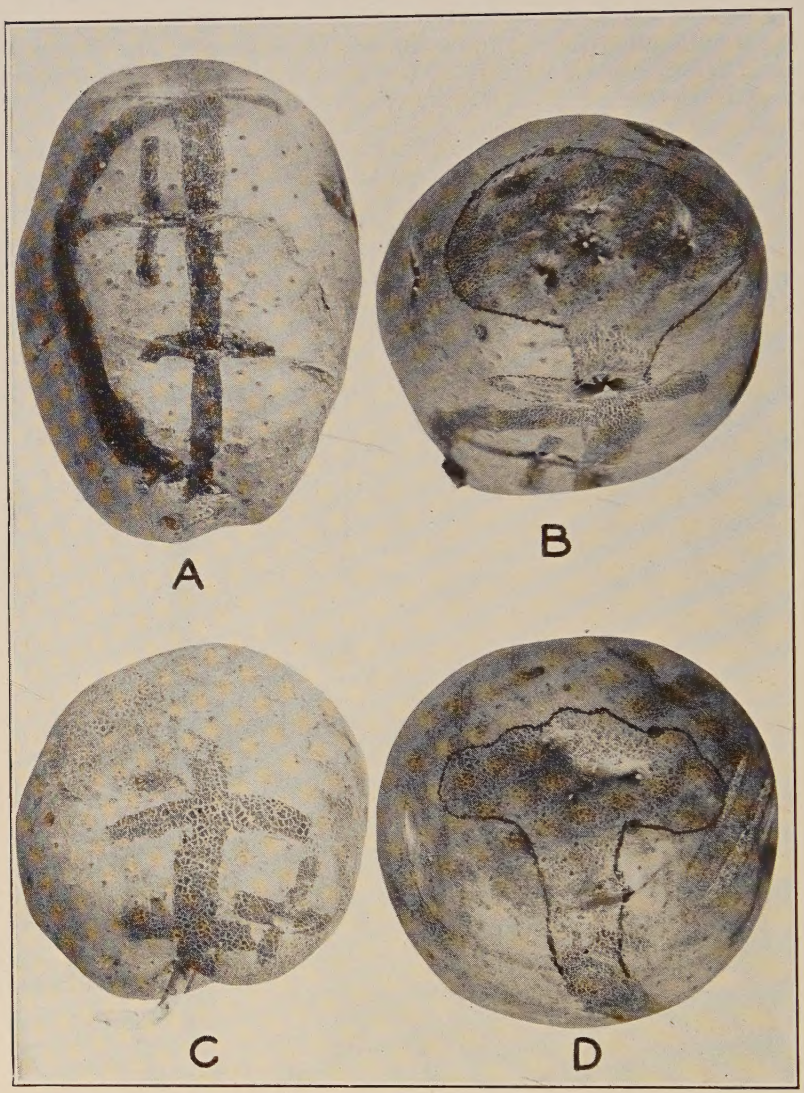


FIG. 1.—A, an elongated type of tuber as exemplified by the Early Ohio variety. B, rounded type of tuber exemplified by the Irish Cobbler variety. C and D, side view of a tuber showing the method of marking growing tubers with India ink. The relative spread of this ink indicates where growth occurs. Plate I shows photographs of certain tubers so marked

RESULTS

It is well to describe the initial stages in tuber formation before discussing the results obtained in connection with the growth studies on tubers of 1 or 2 inches in diameter. Tubers are formed on stolons which are located at the axils of scalelike leaves on underground stems. The first indication of a tuber is a localized swelling of the



Increase in size occurring on various portions of growing tubers as indicated by the diffusion of India ink placed on their surface. The method of marking is illustrated in Figure 1

A.—The ink has not spread on the basal end of a growing elongated tuber, but it has spread on the apical end

B and D.—The apical end of tubers which have grown, following the application of ink. Before growth the ink marks formed a small cross at the apical end; here it is scarcely visible to the eye owing to spread by growth. The apical bud is located near the center of the ink. In order to show more clearly the margins of the original ink marks these were retraced before photographing

C.—Ink has spread on the basal end of the growing round tuber

b
911

stolon, usually at or near the tip. At first this swelling is clavate but soon it becomes rounded and later takes the shape characteristic of the potato variety. At the time when the marks were made on the tubers for growth observation, they had had their characteristic shape for some time.

Enlargement

External enlargement takes place in two general directions as in any growing dicotyledonous stem. There is a general increase in length at the apical end accompanied by an increase in diameter. The criterion for this was the relative degree of ink spread and the direct measurements. The manner of growth for all tubers was not uniform, but was modified by the varietal shape of the tuber. Round tubers increased in size rather uniformly in all diameters, as is indicated by the uniform spread of the ink marks over the entire surface, while elongated forms, on the other hand, grew much more rapidly in length (fig. 2), this growth being much more conspicuous toward the apical end (pl. 1, A and B). In no case where measurements were made did a tuber of any variety grow more rapidly in diameter than it did in length (Table I).

Table I.—*Growth of potato tubers in length and width after varying lengths of time*

Days of growth	Tuber No.	Growth in—		Days of growth	Tuber No.	Growth in—	
		Length	Width			Length	Width
		Mm.	*Mm.*			*Mm.*	*Mm.*
4	1	10	10	7	7	16	11
4	2	18	12	7	8	14	6
				7	9	18	4
	Average	14	11	7	10	10	2
				7	11	11	3
6	3	7	2	7	12	12	4
6	4	11	3	7	13	15	7
				7	14	12	11
	Average	9	2.5	7	15	14	10
7	5	18	12		Average	14	7.25
7	6	14	10				

According to Jürgens (*10*), the form of tubers is governed by the relation of the length growth to thickness growth. Artschwager (*1*) found in his researches on the anatomy of the potato plant that the cells which divide and grow to cause the enlargement of a tuber are not primarily those of the cambium. The first swelling of the stolon is caused by an excessive cell division of the pith up to the time tubers are 5 to 10 millimeters in average diameter. From this time on, the cells of the premedullary zone and, to a lesser extent, those of the cortex are responsible for the increase in size. Reed (*16*) maintains that the pith and premedullary zone contribute equally to the tuber enlargement.

Formation of Cork and Epidermis

The most interesting thing from the standpoint of the scab disease is the changes which occur at the apical end as the tuber grows. The new tissue which is being formed there is always covered by a single layer of epidermis during the stage of rapid tuber development. A short time after the epidermis is formed, depending on the speed

of growth, periclinal walls are formed in its cells and in the subepidermal parenchyma cells. These cells, after several such divisions, are transformed into the corky layer by the suberization of their cell walls and by a gradual loss of their contents. The parenchyma cells lying immediately below the corky cells become the phellogen and supply any additions to the original corky layer.

Lutman (*14*), in connection with his studies on immunity of tubers to scab, made a number of tests to determine the thickness of the corky layer in many varieties of potatoes, but he has made no mention of the nature of the protective covering at the apical end. The result of his work, although suggestive of the fact that the thickness of cork has some bearing on the resistance of tubers to scab, offers no clear-cut evidence in support of such a theory.

As maturity approaches, the tuber continues to increase in size, but less rapidly. At this time the entire, or nearly the entire, surface is covered by a corky layer several cells in thickness. There seems to be a somewhat direct correlation between the rate of

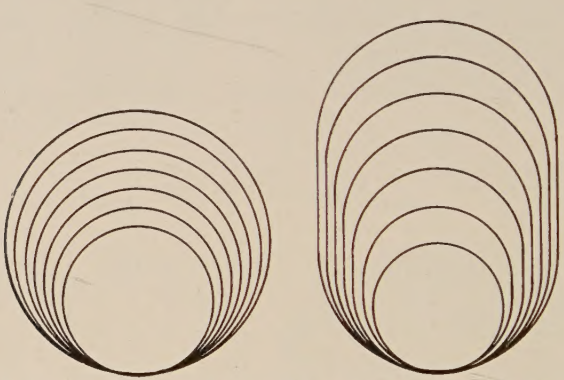
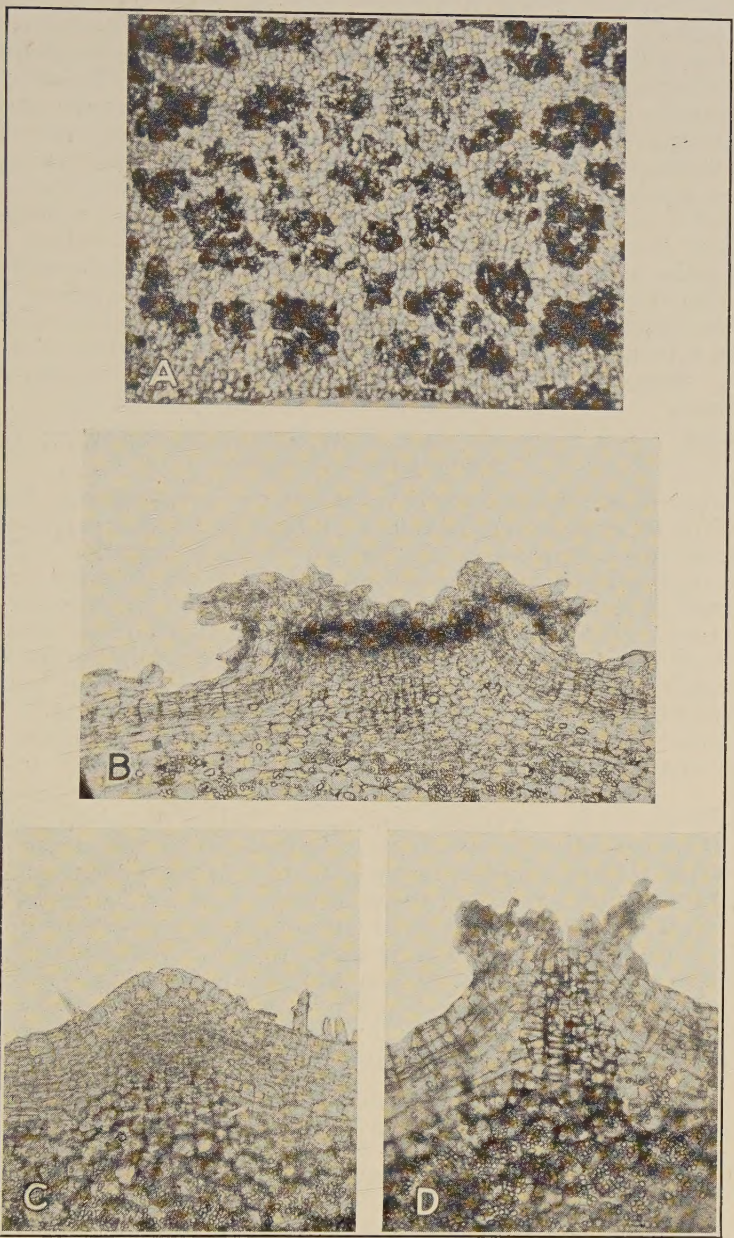


FIG. 2.—Diagram illustrating the method of growth in round and in elongated tubers

growth and the quantity of epidermis at the apical end; the faster the growth the greater the area of epidermis, and vice versa.

FORMATION OF STOMATA AND LENTICELS

In the new epidermis formed at the apical end stomata are found in abundance. Shortly before the new corky layer appears these become somewhat raised on nipplelike protuberances. Judging by the number of increased cells found in the region below the stomata as compared with the number of surrounding subepidermal cells, one is led to believe that the swelling may be caused by a rapid division in this underlying portion, although the writer has not observed division. Below the stoma, between the cells of the swelling, are found large intercellular spaces which appear darkened in fresh unstained sections (pl. 2, A). This swelling continues, and more intercellular spaces are formed until finally the epidermis or the new corky layer, which sometimes begins to appear by this time, is ruptured and a lenticel is formed. This rupture exposes to the surface the free parenchyma cells, with their large intercellular spaces, at the center of the lenticel. The rapidly dividing cells of the subepidermal layer, which are forming cork at this time, are



A.—An enlarged portion of the corky covering of a tuber which had been earlier marked with india ink, this ink appearing as darkened areas with the new cells of the corky layer showing between

B.—An old lenticel after the corky layer of the tuber has been completed. The cells in the darkened portion of the lenticel crater are heavily suberized

C.—The first stage of lenticel formation, a swollen protuberance with a stoma on top and intercellular spaces below. The corky epidermal layer has not yet formed

D.—A lenticel recently broken open. The dividing cells of the subepidermal layer are freely exposed to the surface. The intercellular spaces also extend to the surface

likewise exposed around the ragged external margin of the lenticel (pl. 2, B). If one cuts and examines older lenticels there seems to be a protective layer or pad of empty cells just outside of and in contact with the parenchyma cells below (pl. 2, C). These parenchyma cells having the intercellular spaces are no longer exposed to the surface; this is also true of the phellogen. The cells of the subepidermal layer which were dividing when the lenticel was first formed, have now become cork and are no longer capable of division. Sections of old, completely formed lenticels when treated with Sudan III show this padlike portion within the crater of the lenticel to be of a fatlike nature. This region gives a reddish-orange reaction, as does also the corky layer covering the surface of the tuber. This reaction indicates that the protective layer over the lenticel is suberized in a manner similar to the corky layer covering the rest of the tuber. Jürgens (*10*) claims that all cells of mature lenticels are suberized.

TIME OF TUBER SETTING AND RELATIVE GROWTH OF INDIVIDUAL TUBERS

In the course of some experiments in the greenhouse it became necessary to examine entire hills of tubers at various periods from the time the tubers were first formed to their maturity. Counts made of the number of tubers present at these different periods indicated that all of the tubers were initiated at approximately the same time. These preliminary observations led to further examination to determine the time of tuber setting and the relative growth of individual tubers. Accordingly, 90 pots of Irish Cobbler potatoes were planted and kept in the greenhouse during the spring of 1923. Several of these were emptied at various intervals and observations made on the number, size, and total weight of the tubers on each plant.

Comparisons as to number and relative weight of tubers, must be made between plants which have an equal number of stems. The removal of the extra stems in some pots, in order to make the number of those remaining equal to those in other pots, is not a successful method, for a part of the severed stem often remains below ground, where it continues to live and produce tubers. These extra tubers introduce an error, yet they can not be disregarded, since their development tends to reduce the size of the tubers on the unsevered stems. The difficulties were met by selecting, from a large population of plants those having an equal number of stems.

TABLE II.—*Results obtained from periodic removals of potato hills*

[Potatoes were all planted at the same time]

Days of growth	Number of stems	Number of tubers having an average diameter in millimeters of—															Total number of tubers	Number of hills	Average number of tubers per hill	Average weight of tubers per hill
		1	2.5	5	7.5	10	15	20	25	30	35	40	45	50	55	60				
																				Gram
55	3	22	25	15	18	12	12	4	1								109	5	21.80	14.3
70	3	7	79	77	28	12	2	0	8	3	6	1	2	1			226	9	25.10	69.4
94	3	15	57	36	27	4	7	4	2	2	1	3	2	0	2	3	165	7	23.57	131.0
43	2	25	23	29	13	7	2	1									100	6	16.60	3.0
70	2	8	39	51	14	11	5	4	2	1	0	3	4	0	1	0	143	8	17.87	72.1
94	2	3	22	31	23	12	4	1	1	1	0	0	3	3	3	1	108	7	15.42	139.1

Out of a population of 90 plants, 21 produced three stems and 21 produced two stems. Of the three-stemmed individuals, 5 hills were dug 55 days, 9 hills 70 days, and 7 hills 94 days after planting; of the two-stemmed individuals, 6 hills were dug 43 days, 8 hills 70 days, and 7 hills 94 days after planting. After each digging observations were made on the average diameter of the tubers, the total number of tubers per hill, and the weight of tubers per hill (figs. 3 and 4; Table II).

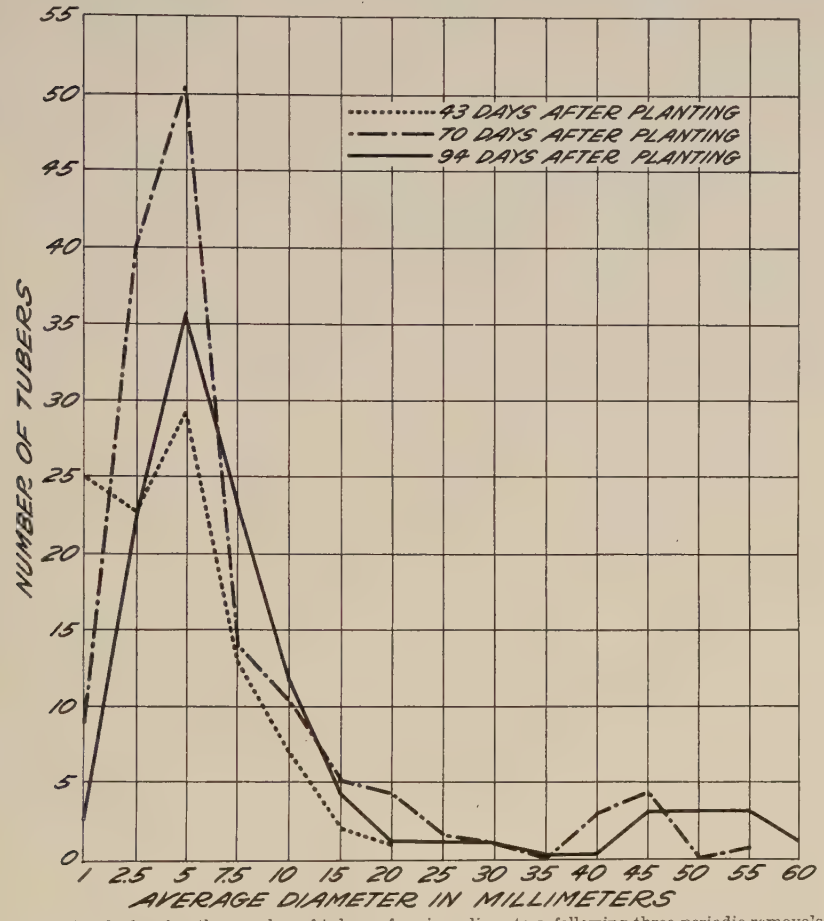


FIG. 3.—Graph showing the number of tubers of various diameters, following three periodic removals of whole hills having two stems each. The majority of the tubers have remained 5 millimeters in diameter, which shows that the smaller tubers have not grown. The larger tubers have increased in size with each consecutive digging

Among the three-stemmed plants, those dug after 55 days showed an average of 21.8 tubers per hill, after 70 days 25.1, and after 94 days 23.4. These figures show an increase for a time in the number of tubers per hill, followed by a decrease. Of the two-stemmed plants, those dug at 43 days after planting showed an average of 16.6 tubers per hill, after 70 days 17.3, and after 94 days 15.4. Thus a slight increase is shown at first, followed by a decrease at the last digging. The last digging of the three-stemmed plants showed a

slightly larger average per hill than the first, but less than the second, while the last digging of the two-stemmed plants showed a decrease in the average number of tubers per hill from the first and second removals. There was a marked increase in weight all through each series (Table II).

The observations noted above, together with the additional data presented in Table II, indicate plainly that all tubers are set at approximately the same time. The average weight of tubers per hill further indicates that growth did occur. The natural question arises: Which tubers grew? Figures 3 and 4, which were made from data on the average size of tubers as coordinated with the number of tubers, throws some light on this subject.

If one considers the plants which had three stems each, it will be seen that at whatever stage of maturity the tubers were dug, the greater number of tubers are 2.5 millimeters in diameter (fig. 4). Now, if the smaller tubers grew appreciably, one would expect these to shift in the graph from the 2.5 millimeters ordinate to one higher; but this does not occur in any of the three series of diggings. The same general principle holds with respect to the plants having two stems each, except that in this case the maximum number of tubers at each consecutive digging had an average diameter of 5 millimeters (fig. 3). This should indicate then that the smaller tubers never grow. Table II shows that the increase in weight was due to the growth of the larger tubers. Those plants dug 55 days after planting each had 12 tubers with a diameter of 15 millimeters, four with a diameter of 20 millimeters, and one with a diameter of 25 millimeters. On the other hand, Table II also shows that those plants which were dug 94 days after planting had one tuber 35 millimeters in diameter, three 40 millimeters, two 45 millimeters, two 55 millimeters, and three 60 millimeters. This increase in size of the larger tubers also holds true for the two-stemmed individuals (Table II). Evidently, then, the increase in weight is due to the growth of the larger tubers and not to the growth of the small ones, which remain nearly stationary in size. If hills of tubers are dug at a time when maturity is approaching, many of the smaller tubers will be found shrunken and disintegrating. This indicates further that some of the tubers which set never grow to any appreciable size, because they are sacrificed to their rapidly growing neighbors, perhaps by resorption of their contents, since they generally shrink without decaying. The relation of this fact to disease will be discussed later.

Clark (4) in his work concludes that the difference in size of tubers in individual hills is to be attributed largely to unequal rate of growth rather than to differences in age of the tubers. His observations are based on fairly large populations grown in the field, and it seems reasonable to believe that his conclusions are correct. Clark further states that there was a slight increase in the number of tubers for a time in the early life of the plant, followed later by a decrease. He attributes this decrease to the shrinkage and disintegration of the smaller tubers; the same conclusion that has been reached in the present work. The evidence seems to indicate that all tubers are set at relatively the same time and that the decrease in number is caused by disintegration during the life history of the plant.

It is not the writer's purpose to discuss at length dominance and inhibition (*3, 12, 15*) from a physiological viewpoint, but simply to mention them and their possible application to the case of growing and nongrowing tubers. In axiate plants, the fact has long been known that a physiologically active growing portion may inhibit the growth of other parts at a more or less remote distance. This has been found true in the case of growing tips, roots, leaves, and other portions of the plant. The removal of these active parts has been shown to allow the dormant parts to resume activity. Various hypotheses have been advanced to account for this phenomenon,

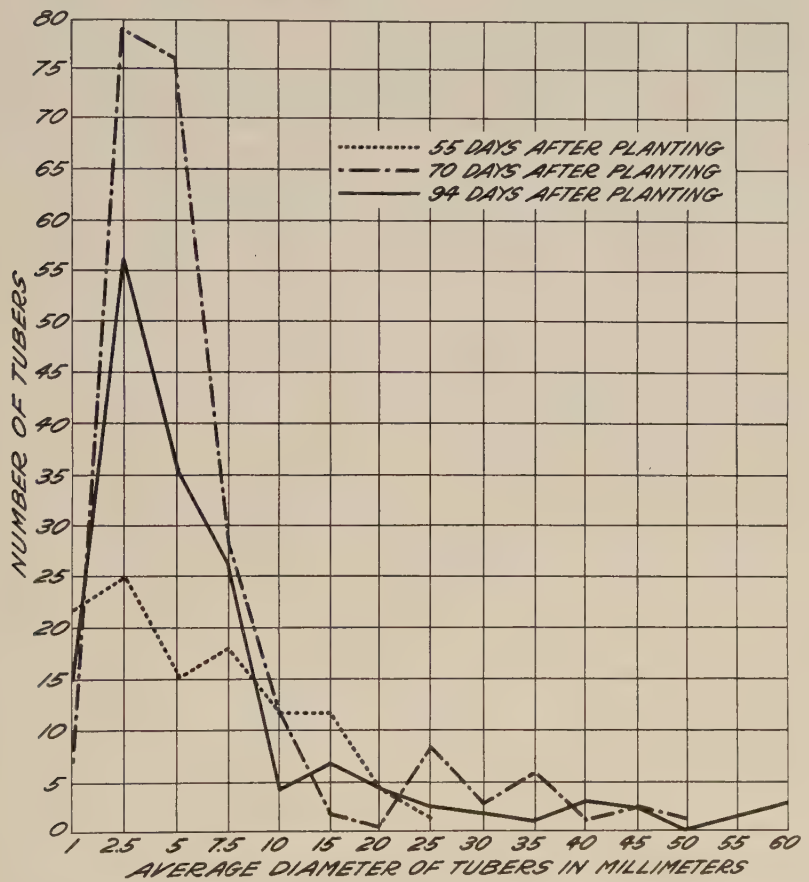


FIG. 4.—Graph showing the number of tubers at various diameters following three periodic removals of whole hills having three stems each. The majority of the tubers have remained 2.5 millimeters in diameter, indicating that the smaller tubers have not grown. The larger tubers have increased in size with each consecutive digging

as for instance, that the growing portion may remove nutritive materials that are necessary for the activity of the dormant parts. It seems plausible to assume that a rapidly growing tuber may inhibit the growth of other tubers in the vicinity. The stolons of these large tubers are scattered up and down the underground stem in such a manner that they are never far removed from the smaller nongrowing

ones. This point should be further investigated by removing some large tubers and determining whether the smaller ones would again resume growth.

RELATION OF GROWTH TO DISEASE

The preceding portion of this paper has discussed entirely matters concerning the development of the potato tuber. The remaining portion will deal principally with the relations existing between the activities previously described and infection of the potato tuber by *Actinomyces scabies*. Especial consideration will be given to the relation of the presence of stomata and the method of lenticel formation to scab susceptibility and infection.

The organism used for all inoculation work was isolated in 1918 by McKinney (*9*) from scabby tubers grown in Door County, Wis. The pathogenicity of the organism has been thoroughly established in a series of greenhouse and field experiments conducted at the University of Wisconsin during the last three years (*9*). Its morphology is the same as that described by Thaxter (*17*) and Drechsler (*5*), and it is therefore recognized as belonging to the species *Actinomyce scabies* (Thaxter) Güssow. The potatoes used were of the Irish Cobbler variety secured from the Spooner Branch Experiment Station, Spooner, Wis.

METHOD OF STUDY

During the course of the experiments the soil temperature was kept at 21° C., which temperature was maintained by the use of the Wisconsin soil temperature tanks (*8*), and the moisture held at 60 per cent of the moisture-holding capacity of the soil. The moisture content was regulated by means of biweekly weighings, and at each weighing water as needed was added through a glass tube inserted in the center of the soil at a depth of about 4 inches. In earlier tests (*9*), it was found that a soil held at 21° C. and having its moisture at 60 per cent of the moisture-holding capacity was favorable both for tuber and for scab development.

The most important part of this work was done by a periodic inoculation process, a method which enables one to inoculate growing tubers at any stage of their development without disturbing them. The seed piece was planted in galvanized iron pots 6 inches in diameter and 10 inches high. An inoculation apparatus was inserted in the can before the soil and seed piece were put in (fig. 5). This apparatus consisted of a hollow spiral lead pipe, the lower end of which was closed and the upper end open. The spirals of the pipe were of such a diameter that when the whole coil was placed in the can it lay snugly against the wall. The upper open end of the coil was allowed to protrude so that the inoculum might be introduced into it easily. The inoculum introduced into the coil was allowed to escape into the soil through small openings at intervals of 1½ inches apart made through the surface of the spiral which faced its axis. The coil was so placed that it surrounded that portion of the soil in which new tubers were formed. The soil used was a mixture of virgin soil and sand. The bottom 3 inches of the can were filled with virgin soil upon which the seed piece was placed,

and the next 5 inches with coarse and fine sand, or a mixture of sand and soil in various proportions. The purpose in using sand was to allow an even distribution of the spores through the soil, for soil of a fine texture seemingly retains the spores and prevents their distribution. All of the sand and soil, as well as the lead pipe and cans, were sterilized in an autoclave for four hours at a pressure of 15 pounds. The lead pipes were coated with paraffin after sterilization to guard against any toxic action which the lead might have on the fungus.

The thoroughness of the spore distribution through the soil was demonstrated by sterilizing a can of soil, in which the lead pipe had been placed, then forcing a spore suspension through the pipe into the soil according to the method described later. Nine portions of the soil, 0.5 cubic centimeter each, were removed under aseptic conditions from the center of the soil mass at depth intervals

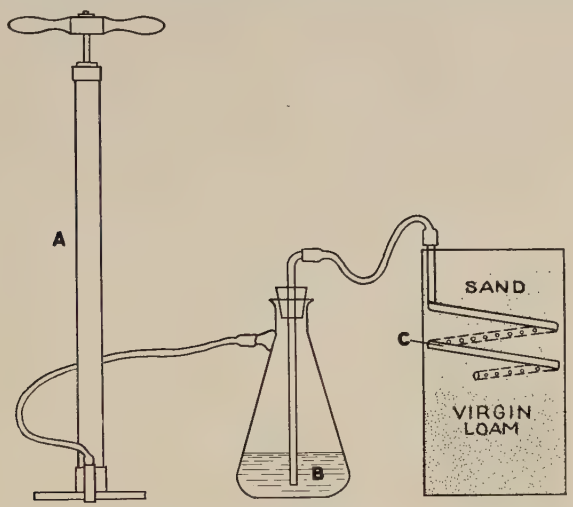
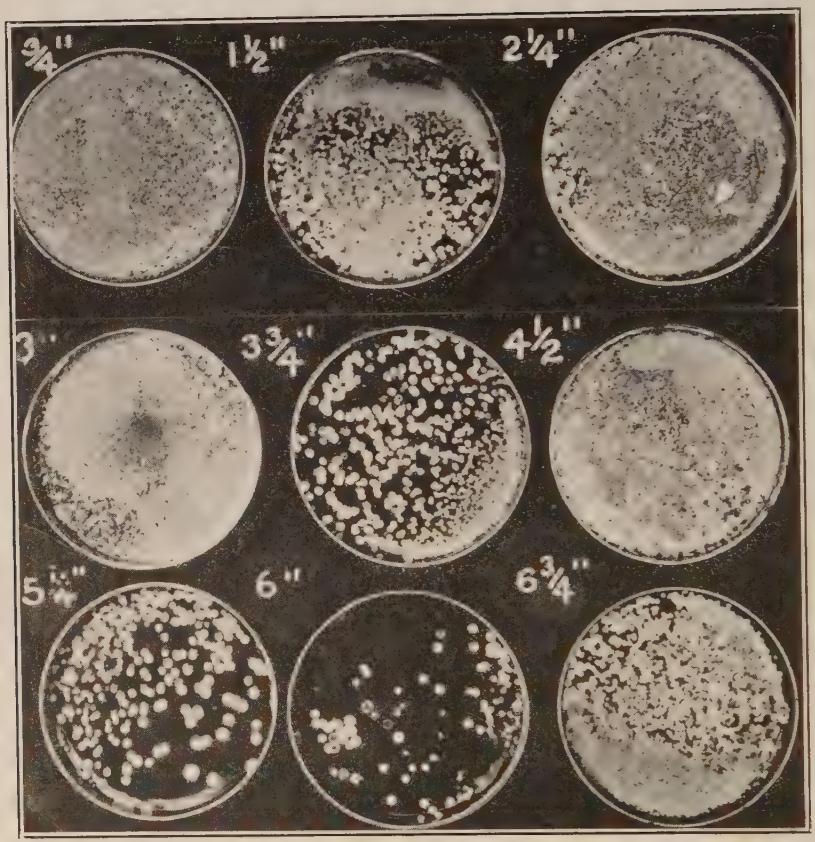


FIG. 5.—Diagram of the apparatus used for inoculating the sand which surrounded growing tubers. The inoculation was brought about without disturbing these tubers. A, large automobile tire pump; B, spores suspension contained in a side-neck filter flask; C, spiral lead pipe surrounding the growing tubers. The holes in this pipe allow a uniform distribution of the spores through the sand in which the tubers are growing

of three-fourths of an inch. Each portion removed was placed in 5 cubic centimeters of sterile water and thoroughly mixed with it. One cubic centimeter of the mixture was poured on the surface of sterile agar in Petri dishes. Plate 3 shows the abundance of the organism in 0.1 cubic centimeter of soil at varying depths. The spore distribution certainly seemed thorough enough to reach all portions of the growing tubers.

In order to secure the organism in abundance for inoculation, it was grown on the surface of potato-dextrose agar in Petri dishes. When sporulation occurred copiously the surface layer was scraped off and made into a spore suspension. Each pot was inoculated with a suspension from the spores of three Petri dishes placed in 300 cubic centimeters of water. After the normal water content of the soil had become somewhat diminished, the 300 cubic centimeters of spore suspension was applied. A rubber tube was attached



The distribution of *Actinomyces scabies* spores through the soil in 10 by 6-inch cans, secured by the spiral pipe method. (See fig. 5.) Samples were taken at ¾-inch intervals from the center of the soil, beginning at ¾ inch depth and continuing to 6¾ inch depth. The colonies on each plate were secured from $\frac{1}{10}$ c.c. of soil. The numbers at the upper left-hand portion of the plates refer to the depth in inches at which the sample was taken

to the projecting end of the lead pipe and connected at the other end to a glass tube which extended through the cork to the bottom of a side-neck filter flask (fig. 5). The side neck of the flask was, in turn, connected by means of a rubber tube to a large automobile pump. The spore suspension was placed in the flask and when the pump was operated it was forced into the lead pipe out through the small holes into the soil. When the young tubers had been set for a short time, a certain number of the pots was inoculated, and at varying intervals of tuber growth, other pots were inoculated. Observations were made on the tubers to determine the length of time required for the disease to develop, the portion of the tuber infected, the place of infection, and the condition of the protective covering.

The experiment in which it was found that tubers set at about the same time, some growing and others remaining relatively stationary, suggested that there should be an inquiry into the relative amount of disease on tubers of different behavior. Seed pieces were therefore planted in 24 cans containing preinoculated soil. Previous to the inoculation, the soil was spread in a thin layer on a concrete floor and a spore suspension of *Actinomyces scabies* was sprinkled over it, the two being occasionally stirred thoroughly. Before this was done, however, the soil had been allowed to dry for some time, and the amount of water used in the suspension was of the right quantity to bring it to the required water content. The tubers were grown at a constant soil temperature of 21° C., and in soil kept at 60 per cent of its water-holding capacity. The relative amount of disease was determined by the number of tubers showing lesions.

In addition to the greenhouse experiments, others were performed in the field to discover whether or not the organism would infect tubers which were growing, but were forming no new epidermis, stomata, or lenticels. After the tubers had nearly reached maturity but were still growing, the soil was removed from around them, and they were marked with India ink and inoculated with a spore suspension. The soil which was replaced upon the tubers was also thoroughly saturated with the suspension. After the tubers had grown, as was determined by the India-ink test, they were dug and observations were made for the presence of the disease and the nature of the tuber growth, especial attention being paid to the condition of the protective covering in all parts of the tuber.

If the development of the disease depends on growing tissues, then the development of the lesion should give some indication of this fact. To determine if any such relation existed, the place of infection and the development of the lesion were carefully observed. Free-hand sections were made of lesions in several stages of development. These were made in an unstained and unkilled condition, so that the browning produced by the organism on the cells might be retained. This browning is lost entirely in the process of killing and embedding, which makes it difficult to trace the effects on the organism when it is located at some distance from the mycelium.

In order to determine if infection could occur on tubers of various sizes which were not growing, 10 tubers, averaging from 8 to 50 millimeters in diameter, were removed from plants and placed in a moist chamber, where they were immediately inoculated with a spore suspension of *Actinomyces scabies*. The surface protective covering varied among the several tubers. Some had an epidermis at the

Results of inoculating tubers with *Actinomyces scabies* after they have reached considerable size. The inoculation was accomplished by means of a special apparatus which prevented any disturbance of the tubers. (See fig. 5)

A.—Side view showing apical-end infection

B.—View of the apical end showing the scabbed portion surrounding a small spot where the disease has not yet become visible to the unaided eye. A microscopic examination showed that the lenticels and stomata of this region were infected

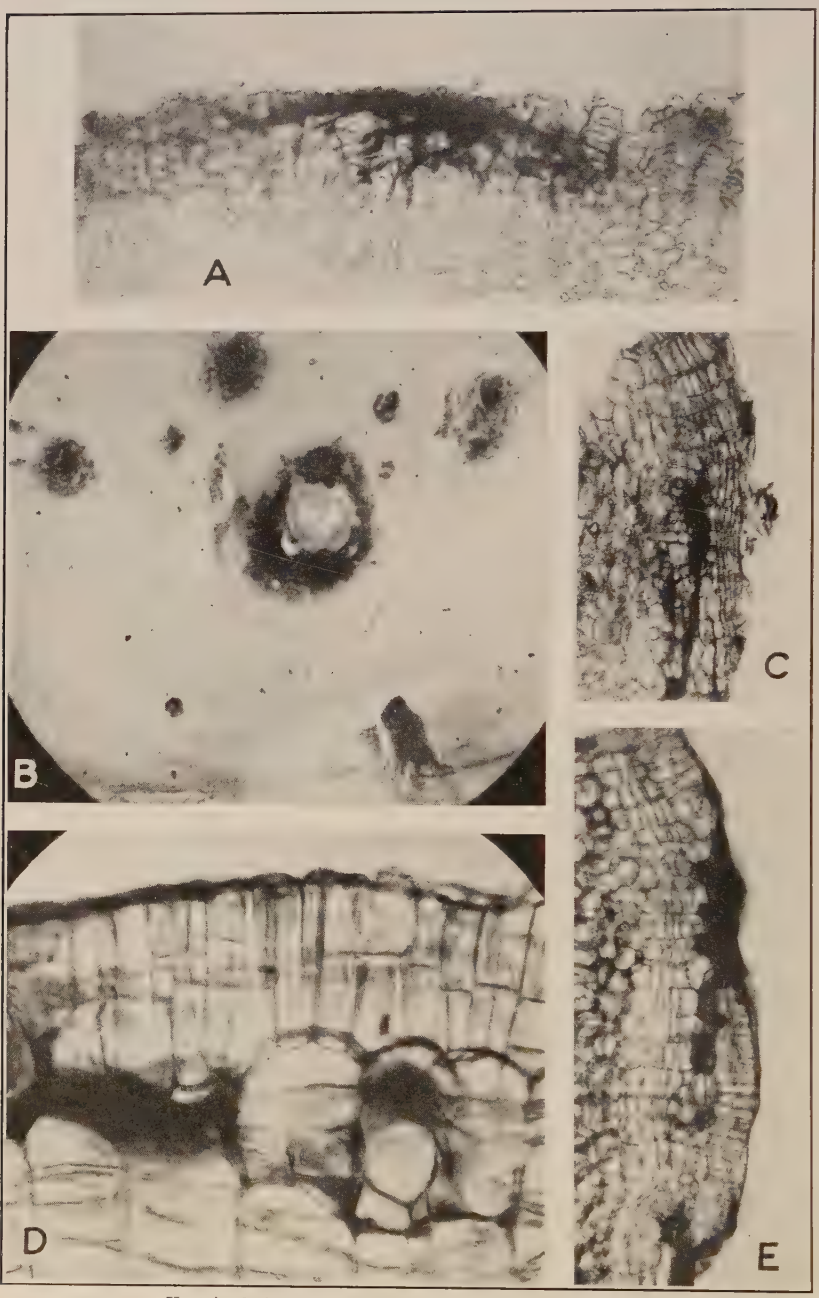
apical end with stomata and new lenticels present, whereas others were entirely covered with cork and had only mature lenticels. The spore suspension was sprayed on the surface of the tubers with an atomizer every other day for a period of two weeks. In no case did infection occur.

RESULTS OF PERIODIC INOCULATIONS

EXPERIMENT NO. 1.—The seed potatoes were planted on April 14, 1922, according to the manner described under "Methods." The first inoculation was made on June 3, 1922, in six cans. Three of these contained pure sand and three contained three-fourths sand and one-fourth soil. The tubers were removed from the six cans 10 days later and the following observations were made: Of a total of 104 tubers removed, the size of which ranged from 46 by 36 millimeters to less than 1 by 1 millimeter, 21 were infected. In no case was a tuber smaller than 10 by 10 millimeters infected, although there were 77 tubers, 80 per cent of the total, smaller than this. The infection was very abundant wherever it occurred, but in every case it was found on the apical end only (pl. 4, A). A noticeable infection of the lenticels occurred in the immediate vicinity of the eyes and the scale surrounding the bud of the eye (pl. 5, B). In a very small area in the region of the fastest growth, i. e., the vicinity of the terminal bud, there was a spot, variable in size, in which no infection was visible to the naked eye (pl. 4, B). A microscopic examination revealed, however, that the stomata there were infected, but the infection was so recent that it had not yet had time to become appreciable to the naked eye. The controls were free from disease.

EXPERIMENT NO. 2.—The potatoes used in experiment 2 were planted at the same time and in the same manner as those in experiment 1. On June 20, 1922, four pots containing one-half sand and one-half soil, and two pots containing three-fourths sand and one-fourth soil, a total of six pots, were inoculated. The removal was made nine days after the inoculation. The main results obtained were very similar to those in experiment 1, that is, all infections were at the apical end and the small tubers were not infected. It should be mentioned here that all of the large tubers in this case were also free from disease. The possible reason for this will be discussed later. The size of the tubers ranged from 50 by 44 millimeters to those smaller than 1 by 1 millimeter. The largest infected tuber was 33 by 23 millimeters. The total number of tubers was 96, the total number infected was 9, that is, 8 per cent of the total. The points to be considered here are that infections occurred only at the apical end and that neither the small nor the large tubers were infected.

The condition of the external protective tissue in various parts of the tubers as found in the two experiments previously described needs further consideration. As regards the protective tissue in the large, uninfected tubers, this was found to be cork, which covered the entire tuber. The stomata were all transformed into lenticels. With respect to the smaller uninfected tubers, various conditions were found. In most cases the tubers were completely corked, with lenticels present on the extreme apical end. In other small tubers,



Unstained photomicrographs of various phases of infection

A.—Cross section through a young lesion. The blackened parts show that the infection has occurred at the surface and has then extended to the interior among the subepidermal and parenchyma cells

B.—An enlarged photograph showing infection of the scale surroun ling the compound bud of an eye and the lenticels in the vicinity. The crescent shaped darkening near the center shows the infection of the scale and the other rounded dark spots near by show lenticel infection

C.—Cross section of the margin of a lesion as seen beneath the surface, showing the spread of the disease along the phellogen region beneath the corky layer. The external portion of the lesion is to the right and out of the photograph

D.—Effects of the organism through the middle lamella of dividing cells in the subepidermal region. Enlargement taken from Plate 5, E. No mycelium is present in this region

E.—Young lesion demonstrating the spread of the disease from the surface portion of the lesion between the dividing subepidermal cells

the basal end was covered with cork, and the apical end with an epidermis. In these instances the outer portion of the epidermal cell walls were heavily cutinized, much more so than in the case of epidermal cells which cover rapidly growing tissues. This would indicate that growth had ceased in these small tubers. The apical

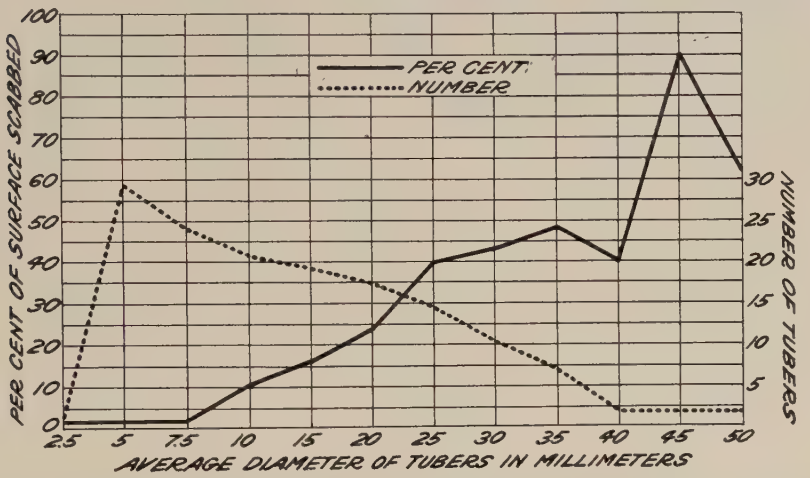


FIG. 6.—Graph showing the percentage of surface scabbed on tubers of various diameters and the number of tubers at each diameter. The percentage of surface scabbed as represented by the continuous line is greater on the tuber of the larger diameter. The tubers represented here were taken from 24 hills of potatoes the tubers of which had made their entire development in the presence of *Actinomyces scabies*

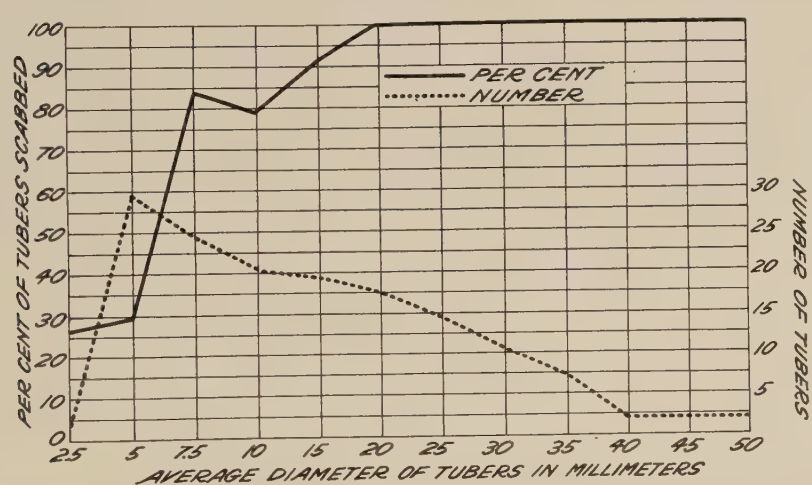


FIG. 7. -Graph showing the percentage of tubers of various diameters which have become diseased and the number of tubers at each diameter. The percentage of tubers scabbed as represented by the continuous line is greater among tubers of larger diameters. The tubers represented here were taken from 24 hills the tubers of which had made their entire development in the presence of *Actinomyces scabies*

end of infected tubers was covered with an epidermal layer in which were found stomata and newly formed lenticels.

In the case of tubers grown in preinoculated soil, there was a great difference in the percentage of the surface scabbed in the large and the small tubers. This was even more pronounced when one considers the percentage of tubers scabbed rather than the percentage

of surface scabbed. On the basis of the percentage of surface scabbed, if one compares tubers having an average diameter of 5 millimeters to those having an average diameter of 35 millimeters, it will be found that the percentage varies from 2 to 51 (fig. 6). On the basis of the percentage of tubers scabbed, considering the same sizes of tubers mentioned above, the percentage ranges from 25 to 100 (fig. 7). All of the tubers from 20 millimeters in diameter to the largest were infected. The reason why tubers smaller than 5 millimeters and larger than 35 millimeters were not considered is that the population was not thought large enough to be representative. The omitted ones, however, showed the same tendency as the others.

RESULTS OF FIELD INOCULATIONS

In the case where tubers were inoculated in the field no disease resulted either on the growing or on the nongrowing tubers. Field work presents the great difficulty that after the tubers are dug around, a large number of them fail to grow. This failure may be accounted for in two ways. At the point where the stolon of a tuber joins the underground stem there are a large number of roots united to the underground stem in such a way that their points of attachment partially surround the point where the stolon is attached at the node. This makes it difficult to remove the soil from around a tuber without destroying the roots, not only of those having their origin near the stolon, but also of some arising from other nodes. On the other hand, it might be that the particular tuber under observation had already reached its maximum size and would not have grown any more even if allowed to remain undisturbed. In all, 40 tubers were inoculated and marked with India ink to determine whether or not they grew. Of this number, 14 increased in size. On examination it was found that the method of growth was simply by a swelling of the tuber without the formation of any new epidermis or stomata at the apical end, a condition contrary to that found in younger growing tubers. It should be remembered, however, that the tubers used in this experiment were nearly mature, as was mentioned under "Methods."

POINT OF INFECTION AND FORMATION OF LESIONS

In order to determine with any degree of certainty the point of infection, it is necessary to detect the infection in its very early stages, for if it has started in the stoma or lenticel, and progressed for any apprecialbe time, all signs of these organs will be obliterated. Wherever it was possible to distinguish the point of infection, it was found in a lenticel or stoma, except where infection occurred near an eye, in which case it was always in a region where the protective layer was epidermis and stomata were present in the vicinity. Lutman (*13*) claims that the principal method of infection is through lenticels, but that it can also take place through the corky layer. As mentioned before, one must detect the infection very early or he will be deceived as to its exact location. A surface view of a newly infected lenticel usually shows a few darkened cells all around the periphery of the lenticel; in some cases the lesion may start at one edge and extend out from the side in a more

or less rounded form. This darkened area continues from the margin of the lenticel out under the external corky covering. One can see from the surface view the somewhat transparent corky layer over the darkened tissue beneath. A newly infected stoma appears normal as far as shape of the guard cells is concerned, but a light brownish color may be noted in the opening of it, and this extends along the margin of the guard cells and the cells adjacent.

A critical examination of fresh, unstained sections beneath the surface of the tissue immediately next to a young lesion reveals some interesting details of the manner in which the disease spreads from the original point of infection. The phellogen and subepidermal cells, which are regions of rapid cell division, especially during the formation of new cork, are blackened for some distance from the lesion as seen from the surface (pl. 5, A, C, E). A minute inspection of the details of the blackened cells shows that the extension takes place through the middle lamella. At the angles where four cells have come in contact they separate from one another and the space between soon becomes filled with a dark-brown liquid. A dark-brown color extends out along the middle lamella, which becomes abnormally thickened (pl. 5, B). Occasionally one finds a whole cell blackened, a condition which is caused perhaps by the darkening of the middle lamella of the wall which presents its face toward the observer.

A considerable amount of search has failed to reveal threads of the mycelium in the vicinity of the blackened tissue, a fact which might lead one to believe that the causal organism affects cells at a considerable distance. Bolley (*2*) claims that he found the mycelial threads of *Actinomyces scabies* in the diseased tissues. Others have stated that they have seen short, rodlike bodies present in the cells, and these they have believed to be broken fragments of the mycelium. In no case does the literature state whether or not the specimens were artificially inoculated in sterile soil. The writer has been able to find abundant filaments resembling those of *A. scabies* in the cells of field specimens which were not artificially inoculated. The filaments were not found in living cells but in dead corky ones near the surface of lesions. The lesions were about 5 millimeters or more in diameter. The invaded cells were not empty of food, for starch grains were present. Conclusions should not be drawn from field material, however, for there are many species of Actinomyces in the soil which may penetrate the dead or dying tissue and thus deceive the observer.

Freshly dug diseased tubers show the organism on the surfaces of lesions as a white hyphal covering. This may occur on field or on artificially inoculated specimens. Since the organism has not been found in the cells of diseased tissue, it must live at the surface and extend its influence beneath by means of an enzyme or toxin. The question as to the exact manner by which the organism secures nourishment has not been satisfactorily answered by these studies. It would be at variance with the known physiology of fungi to suggest that suberin might be utilized for nourishment. The walls of the corky cells in a scab lesion disintegrate and allow the cells to slough away, and by this process may permit the organism to gain access to starch grains and old dried cell contents which are found in corky cells.

DISCUSSION

The relation of disease and infection to the behavior of the tuber during the various phases of its life history needs further explanation. The most important points to be considered are: Infection of the tuber at the apical end; relation of tuber growth to disease; and period of susceptibility to infection.

Rapidly growing tubers which were inoculated became diseased only on the apical end, an indication that the conditions which exist in the external protective covering at the growing apical end are necessary for infection. Some of the conditions which have been noted at the time of infection are the presence of an epidermis, or an incompletely formed corky layer, and the formation of natural openings in these coverings. Since infection has always occurred either in stomata or in lenticels while they were in the process of formation, it is reasonable to suppose that these openings are necessary for infection. Stomata and lenticels are formed only in new and rapidly growing protective tissue which might lead to the conclusion that the presence of an epidermis with the formation of such organs in it is the chief factor necessary for infection.

The condition of the lenticels in the apical end of a tuber at the time of infection may predispose the tuber to infection in that region. When the lenticels first break open, the parenchyma cells below are freely exposed. This enables the organism or its products to come into direct contact with these cells. The parenchyma cells thus exposed have not been found to be suberized.

The results obtained in a portion of the experiment suggest that growth is essential for infection. Large tubers exposed to the scab organism during their entire life history showed much greater infection than smaller ones of the same age under the same conditions. Another experiment indicated that the large tubers grew and the smaller ones remained relatively stationary. It appears therefore that growth and disease are coincident.

The inoculation of entire hills of tubers at various periods in their life history resulted in a varying percentage of disease on tubers of different sizes, the larger ones showing some infection and the smaller ones none. There is evidence, as indicated above, that the large tubers were growing and the smaller ones were not. The idea that the occurrence of the disease depends on growth or the ability of a tissue to resume growth readily, is further strengthened by the fact that the tissue in the vicinity of the eyes is often infected. This tissue is able to resume growth very readily. The knobby outgrowths frequently observed on tubers are initiated at the eyes, a fact which shows that this region responds to the stimuli which cause cell division.

There are instances where growth occurs without liability to infection. None, for example, has been observed at the basal end of rapidly growing tubers when inoculated with *Actinomyces scabies*. It often happens that no infection occurs anywhere on the surface of growing tubers, and it never occurs on those not growing.

The condition existing in the external coverings of the tuber at the points where infection failed to occur must, therefore, prevent infection. The protective covering over the immune areas of growing tubers has always been found to consist of several layers of

corky cells similar to those on a mature tuber. Mature lenticels occur in the corky protective coat, but no stomata. The condition of the lenticels prevents infection occurring through them. This lack of infection in mature lenticels may be accounted for by the fact that the tissue covered by the lenticels is protected by a layer of suberize cells. The protective layer in the external portion of the lenticel is several cells in thickness. These facts show that increase in size alone will not predispose the tuber to disease, although it is coincident, in many cases, with the production of the disease. Nongrowing tubers have never become infected when inoculated, even though portions of their surface were covered with an epidermis containing lenticels and stomata. Thus it would seem that enlargement is essential to the production of the disease.

The development of a scab lesion indicates that the presence of dividing cells, or cells readily stimulated to division, aid in the production of the disease. The pathogen first affects the host by extending its influence through the middle lamella of the subepidermal cells. Later, when the host is more mature, it extends through the phellogen. The cells of the subepidermal layer are in a state of active division when the new corky layer is being formed, and at this time infection is very abundant. The cells of the phellogen divide to form new additions to the corky layer. Cell division in the phellogen is easily initiated at any time that stimulating agents are applied. *Actinomyces scabies* stimulates cell division, as is indicated by the type of disease which it produces, for this consists in an abnormally large production of corky cells.

It appears from the experimental evidence at hand that tubers of the size found on the market have at some time in their life history passed through a period of susceptibility, which was later followed by a period of immunity. The period of susceptibility may begin at any time in the growth of the tuber, provided it is forming a new epidermis with stomata and lenticels. The period of susceptibility ceases in growing tubers when the latter are no longer forming an epidermis with stomata or young lenticels, and also in any tuber when it has ceased to grow.

SUMMARY

Recent investigations of potato scab have given evidence that the development of the disease is intimately connected with the growth of the tuber.

Experiments conducted to determine the method of tuber enlargement showed that enlargement varied with the variety. Tubers of varieties that were normally elongated increased in length more rapidly than in width, whereas those that were normally round increased rather uniformly in all diameters.

The apical end of a rapidly growing tuber was found to be the region of greatest growth activity. This portion of the tuber was covered with an epidermis in which stomata were being formed. These stomata later were transformed into lenticels by a breaking open of the epidermis or of the new and incompletely formed corky layer in which the stomata were present. This rupturing of the protective covering freely exposed the parenchyma cells below the lenticels.

The epidermal and subepidermal cells covering the apical end of a growing tuber became transformed into several layers of corky cells by periclinal cell divisions, followed or accompanied by the suberization of the cell walls. The cells of the lenticels present in the corky layer also became suberized, thus protecting the parenchyma cells below.

Tubers produced by a single plant are initiated at nearly the same time. Some of these grow; others remain of approximately the same size. The tubers which remain small often disintegrate, so that a mature plant has ordinarily fewer tubers than a younger one.

The organism used for inoculation was a strain of *Acitinomyces scabies* (Thaxter) Güssow isolated from scabby potatoes grown in Door County, Wis. Its pathogenicity had been thoroughly established before these experiments were undertaken. Its morphology corresponded to that given by Thaxter and Drechsler.

Whole hills of tubers were inoculated periodically at varying stages of maturity by a special apparatus which permitted inoculation without disturbing the tubers or plants. These tubers were grown at a soil temperature of 21° C., and the soil was kept at 60 per cent of its moisture-holding capacity.

Tubers which were inoculated when the plants were only partially mature showed two types of behavior: (1) The large ones were infected at the apical end only and in the vicinity of buds situated near the apical end; (2) the small tubers were not infected.

Tubers which were inoculated when the plants were nearly mature showed three types of behavior: (1) The small tubers were not infected; (2) the medium-sized tubers were infected at the apical end only; (3) the largest tubers were not infected.

Individual tubers growing in the field, which were nearly mature when inoculated, did not become infected even though growth had not ceased.

In all cases where it was possible to determine the place of infection, it was found to be in a lenticel or in a stoma.

In every instance where tubers were infected as a result of artificial inoculation, the protective covering in the newly infected part was found to be epidermis or newly formed cork, in which stomata or young lenticels were being formed.

In every instance where large, nearly mature tubers failed to become infected when artificially inoculated, the entire, or nearly entire, external covering was found to be cork with no new natural openings being formed.

Small tubers which failed to become diseased when inoculated were either completely covered with cork or the basal end had a corky protective coat and the apical end a heavily cutinized epidermis with stomata and lenticels present. These tubers were not growing.

Histological studies of the lesion showed that the effect of the organism extends along the phellogen of the cells of the subepidermal layer by way of the middle lamella which was thickened and darkened. The phellogen and subepidermal layers are regions of rapid cell division in growing tubers.

The mycelium of the causal organism has not been found in lesions produced by artificial inoculation. The mycelium of a fungus resembling *Actinomyces scabies* has been found in lesions produced naturally in the field.

It seems that if the potato-scab disease is to occur, the tuber must be increasing in size, there must be stomata or young unsuberized lenticels present through which the infection can take place, and there must be dividing cells or cells which can easily be incited to division by the products of the organism, thus permitting the production of the typical corky scab lesions.

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